Journal of Controlled Release 279 (2018) 1–7

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Efficacy of paclitaxel/dexamethasone intra-tumoral delivery in treating orthotopic mouse breast cancer

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ARTICLE INFO

Keywords:
Intra-tumoral implant
Local anti-cancer treatment
Promoter drugs
Orthotopic model of breast cancer
Paclitaxel
Dexamethasone

ABSTRACT

The effect of topical co-administration of promoter drugs with paclitaxel to increase anti-tumor effects of paclitaxel was investigated. Mice with orthotopic 4T1-Luc breast cancer received single intra-tumoral injection of a polymeric formulation with paclitaxel and a specific promoter drug. Several promoter drugs were evaluated, including: dexamethasone, losartan, nicotinamide, Azone, and oleic acid. Dexamethasone exhibited the highest effect on paclitaxel anti-tumor activity, in a dose-dependent fashion. However, this effect was accompanied by systemic effects of dexamethasone, and inability to prevent tumor metastasis to the lungs. Topical co-administration of promoter drugs with anti-cancer agents can enhance their anti-tumor effects. Further investigations are needed to identify the most efficient combinations of promoter and anti-cancer drugs, and their suitability for the clinical management of the breast cancer disease.

1. Introduction

The use of anti-cancer agents is suboptimal due to their low clinical efficiency and high incidence of adverse effects. Following systemic administration of anti-cancer agent, a small fraction of it will reach the solid tumor, and even smaller amount will penetrate the internal parts ("deep layers") of the tumor and reach the target cells. As a result, in the tumor tissue, only the cells that are close to the blood vessels will be exposed to the therapeutic concentrations of the anti-cancer drug, while the bulk of the tumor will not be affected by the treatment [1–3].

Over the last two decades, drug delivery systems (DDSs) have been designed to overcome this pharmacokinetic limitation of anti-cancer agents and to improve the balance between their efficacy and toxicity. Many of these DDSs are based on nanoparticles that encapsulate the drug or polymer conjugates [4] that reduce the volume of drug distribution (and limit the exposure of non-target tissues to the drug, and improve its safety profile), prolong its half-life (and improve effectiveness), and are expected to enhance the exposure of the tumor to the drug (via the enhanced permeability and retention effect, EPR). EPR efficiency, however, has been overestimated [4–7]. A recent meta-analysis of data from 232 clinical studies revealed that on average only 0.7% of the systemically-administered nanoparticles accumulated in solid tumors [8]. Moreover, tumor accumulation of DDSs is highly variable in different types of cancer and in different patients with the same diagnosis, and is accompanied with even less efficient drug penetration to the "deep layers" of the tumor, as compared to the free drug [9–12]. More efficient and safe approaches of drug delivery to the tumor are needed to overcome the pharmacokinetic limitations of anti-cancer agents and to improve the balance of their desired effects vs. adverse effects [4–6].

Implantable formulations can deliver high doses of anti-cancer agents directly to the intended site of action in the tumor with minimal systemic exposure and adverse drug effects [13]. For instance, paclitaxel containing semi-solid implants based on the [poly(sebacic acid-*co*-ricinoleic acid ester anhydride)] polymer were extensively investigated in several animal models of cancer disease. Focal (topical) administration of these formulations increased the survival rate, reduced the tumor rate growth, and prevented dissemination of metastases to the lungs and lymph nodes of the treated animals [13–17]. Other types of implantable formulations have undergone clinical studies and were approved for clinical use, for example polymer-based Gliadel® wafers that release carmustine for clinical management of malignant glioma [18].

Overall, implantable formulations can deliver high doses of anti-cancer agents to the desired location. The majority of the organs and tissues are accessible for such administration, including such "difficult"

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https://doi.org/10.1016/j.jconrel.2018.04.010
Received 10 December 2017; Received in revised form 30 March 2018; Accepted 6 April 2018
Available online 11 April 2018

locations as the brain. Moreover, tight control of the drug release is possible by the choice of the polymer composition and properties [18–20]. Despite these advantages, however, focal administration of anti-cancer agents exhibits low clinical effectiveness. For instance, Gliadel® wafers extend survival of malignant glioma patients by only 2.5 months on average, and do not cure the disease [21,22]. Detailed analyses of the drug biofate following focal administration reveals that this finding is due to the limited intra-tumoral drug distribution, as only a thin layer of cells in immediate vicinity to the implant is exposed to the therapeutic concentrations of the anti-cancer drug [23]. Therefore, there is a need to improve the intra-tumoral pharmacokinetics of implantable anti-cancer formulations to enhance the therapeutic permeability of the administered drug and effectiveness of the treatment [15].

Promoter drugs, drugs that improve exposure of tumor cells to anti-cancer effector drugs via enhancement of their tumor uptake and distribution [24,25], can increase effectiveness of anti-cancer treatments. Different types of promoter drugs vary in the mechanism of their pharmacological effects, and the balance of their effects in the tumor and other tissues. For instance, systemic anti-angiogenic treatment (e.g., with bevacizumab, Avastin®, an antibody that binds to vascular endothelial growth factor (VEGF)), can inhibit tumor angiogenesis and enhance effects of "classical" anti-cancer drugs [26] and DDSs [27,28]. Other types of promoter drugs affect the extracellular matrix of tumors, local blood pressure and fluid drainage, permeability of the cell membranes and barriers, etc. [11,25]. For instance, they affect the tumor microenvironment by modulation of inflammation (dexamethasone) [29–31], reduce of stromal collagen (losartan), reduction of interstitial fluid pressure (IFP) and microvascular pressure (collagenase) [25], lowering arterial blood pressure and tumor IFP (nicotinamide) [25], via topical penetration enhancement (Azone, oleic acid) [32–34], etc.

It should be noted that the pharmacological effects of promoter drugs can be complex (e.g., due to pleiotropic pharmacological effects of dexamethasone [31], which include also direct effects on the cancer cells, as well as systemic immune- and metabolic activities; see also below), and can differ between the tumor tissue and other tissues (e.g., for losartan, which is used clinically due to its hypertensive effects [35]). Therefore, the balance of the desired vs. adverse effects of the promoter drugs is expected to be different if they are administered systemically, or targeted to the tumor tissue.

We hypothesized that co-delivery of promoter agents along with an anti-cancer agent directly to the tumor will enhance their efficacy and minimize systemic toxicity. The objective of this study was to assess the effect of promoter agents that can affect the intra-tumoral distribution of anti-cancer agents on the efficiency of intra-tumoral anti-cancer implants, in an animal orthotropic model of breast cancer.

We have investigated implants based on injectable poly(sebacic acid-*co*-ricinoleic acid ester anhydride) loaded with paclitaxel and different promoter agents. Our investigation focused on dexamethasone, which demonstrated the most significant effects, as compared to the other agents that we screened in the initial studies (losartan, collagenase, nicotinamide, Azone, oleic acid; which did not enhance the anti-cancer effects of paclitaxel, or enhanced them only to a low extent).

In this study, we applied an animal orthotropic model of breast cancer based on injection of 4T1-Luc murine mammary carcinoma cells to the mammary gland of experimental mice. This model is similar to human breast cancer with regard to high tumorigenicity with spontaneous metastases. This allows close and detailed monitoring of the disease and its treatment using non-invasive methods, including imaging of the luciferase activity of the cancer cells at the primary tumor and at the metastatic sites [36,37].

2. Materials and methods

2.1. Materials

Sebacic acid (SA), castor oil, dexamethasone, MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), picric acid, formaldehyde 37% w/v solution and Tween® 80 were purchased from Sigma-Aldrich Ltd. (Rehovot, Israel). Ricinoleic acid (RA, purity > 98%) was isolated from castor oil by fractional precipitation using the salt-solubility methods. Paclitaxel GMP grade was obtained from Bioxel Pharma Ltd. (Quebec, Canada). D-luciferin was purchased from Perkin Elmer (Waltham, MA, USA). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate-buffered saline without calcium and magnesium (PBS), fetal bovine serum (FBS), penicillin/streptomycin and L-glutamine were purchased from Biological Industries Ltd. (Beit Haemek, Israel). All solvents used and glacial acetic acid were of analytical grade. They were purchased from Bio-Lab, Ltd. (Jerusalem, Israel) and were used without further purification.

2.2. Preparation of the formulations

Poly(sebacic-*co*-ricinoleic anhydride) at a 3:7 w/w ratio (p(SA-RA) 3:7) was synthesized by insertion polycondensation as previously described [38]. The polymer was characterized by GPC, FTIR, and ^{1}H NMR. Formulations of paclitaxel at 5, 10 and 20% w/w, with or without dexamethasone 10% w/w, were prepared by mixing the drug powders in p(SA:RA) 30:70 using mortar and pestle, at room temperature. The generated formulations were homogeneous, and did not contain visible particles.

2.3. In vitro drug release from formulations

The release studies were conducted as previously described [13]. Briefly, 50 mg of the pasty formulation were placed at the bottom of 50 mL centrifuge tube, loaded with a 50 mL release buffer (0.1 M phosphate buffer solution pH 7.4 with 0.1% w/v Tween 80), and were kept at 37 °C with constant shaking (50 RPM). At the pre-specified time points, samples of the solution were collected, filtered via 0.22 μM filter, and stored at -20 °C pending analysis. The withdrawn volume was replaced with fresh buffer.

Concentrations of paclitaxel and dexamethasone in the collected samples were determined by HPLC using calibration curves. The chromatographic system consisted of HP 1100 HPLC (Hewlett Packard, Palo Alto, CA, USA) interfaced with an HP ChemStation, and a LiChrospher® 100 RP-18 (5 μm) reverse-phase column (Merck, Darmstadt, Germany). For dexamethasone, the mobile phase was composed of acetonitrile and double distilled water (adjusted to pH = 2.3 with phosphoric acid) 43:57 v/v at a flow rate of 1.2 mL/min, and UV detection at 254 nm [39]. For paclitaxel, the mobile phase was composed of acetonitrile and double distilled water (adjusted to pH = 2.3 with phosphoric acid) 65:35 v/v at a flow rate of 1.0 mL/min, and UV detection at 230 nm.

2.4. Cell culture

4T1-Luc cells, murine mammary adenocarcinoma transfected with firefly luciferase, were generously donated by Prof. Ron N. Apte (The Shraga Segal Department for Immunology, Microbiology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel). The cells were kept in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. The cells were maintained in an incubator at 37 °C in a humidified atmosphere with 5% CO_2.

2.5. Cell viability assay

4T1-Luc cells were plated on 96-well plates (10^3 cells/well) and